New insights into urinary acidification and regulation of acid-base balance

Wagner, Carsten

New insights into urinary acidification and regulation of acid-base balance

Carsten A Wagner

Institute of Physiology, University of Zurich, Switzerland

Address for correspondence

Institute of Physiology
University of Zurich
Winterthurerstrasse 190
CH-8057 Zurich
Switzerland
Email: Wagnerca@access.uzh.ch
Phone:+41-44-63 55023
Fax: +41-44-63 56814

Summary

Systemic acid-base balance is primarily controlled by the renal excretion of acids and the exhalation of CO_2 and both processes are tightly regulated and coordinated. Acid excretion into urine requires the formation of ammonium and its transport into urine as gaseous ammonia. Until recently it has been believed that NH_3 would move across membranes by free diffusion according to Overton's rule. Recent structural, functional, and in vivo data show now that Rhesus proteins act as gas channels for NH_3 and mediate renal acid excretion. Lack of the renal isoform RhCG in mice causes reduced ammonium excretion and metabolic acidosis.
Breathing and exhalation of CO_2 is stimulated and regulated by CO_2 and acid sensors in the carotid bodies and the brain stem. GPR4 belongs to a small subfamily of G protein-coupled receptors and is activated by extracellular protons. Mice lacking GPR4 develop respiratory acidosis and are not able to increase ventilation appropriately in response to high CO_2 levels or systemic acidosis. Thus, RhCG and GPR4 present novel paradigms of membrane proteins involved in controlling and regulating systemic acid-base balance in the major organs involved in this task.

French summary

L'équilibre acide-base systémique est principalement contrôlé par l'excrétion rénale d'acides et l'élimination du CO_2 par l'expiration. Ces deux processus sont étroitement régulés et liés. L'excrétion d'acide dans les urines nécessite la formation d'ammonium (NH_4^+) et son transport dans les urines sous sa forme gazeuse, l'ammoniac (NH_3). Jusqu'à récemment, on estimait que NH_3 se déplaçait à travers les membranes par diffusion libre selon la règle d'Overton. Des données récentes, structurales, fonctionnelles et *in vivo* montrent maintenant que les protéines Rhésus agissent comme des canaux à gaz pour le NH_3 et permettent l'excrétion rénale d'acide. L'absence de l'isoforme rénale RhCG chez la souris entraîne la diminution de l'excrétion rénale d'ammonium et l'installation d'une acidose métabolique.

La respiration et l'expiration du CO_2 est stimulée et régulée par le CO_2 lui-même et les capteurs d'acide dans le corps carotidien et le tronc cérébral. GPR4 appartient à

une petite sous-famille des récepteurs couplés aux protéines G et est activé par les protons extracellulaires. Les souris invalidées pour GPR4 développent une acidose respiratoire et ne sont pas en mesure d'augmenter leur ventilation de manière appropriée en réponse à des niveaux élevés en CO_2 ou lors d'une acidose systémique.

Ainsi, RhCG et GPR4 représentent de nouveaux paradigmes des protéines membranaires impliqués dans la régulation de l'équilibre acide-base systémique dans les principaux organes voués à cette tâche.

Introduction

Systemic acid-base balance is the sum of acids and bases circulating in extracellular fluids such as protons, organic acids, bicarbonate and CO_2, phosphate, and protein buffers. In a normal and healthy organism, the sum of these acids and bases results in a pH of approx 7.40, 25 mM bicarbonate, and approx. 38-40 mm Hg pCO_2 in arterial blood. This acid-base equilibrium is critical for normal organ and cellular functions, and deranged acid-base status presents an important risk factor for higher morbidity and mortality in the setting of many diseases as well as when occurring alone.

Acid-base homeostasis is constantly challenged by dietary intake, cellular metabolism, physical activity, and during disease. Consequently, many organs contribute to the maintenance and regulation of systemic acid-base balance including skeletal muscle, liver, the gastro-intestinal tract, and particularly kidneys and lungs/ventilation. Lung ventilation regulates CO_2 levels in arterial blood whereas the kidneys control proton, bicarbonate, and phosphate concentrations in extracellular fluids.

Renal acid excretion

The kidneys excrete about 70 mmoles of acids/ day. Only a small portion is excreted as free protons but most acid is in the form of ammonium (about 2/3) and titratable acids (about 1/3) such as phosphate. Type A intercalated cells along the collecting duct mediate the removal of acids (i.e. protons and ammonium) as well as the generation of bicarbonate [1]. H^+-ATPases localized at the luminal membrane of type A intercalated cells excrete protons [2] thereby acidifying urine. However, H^+-ATPases can generate only a maximal pH gradient of about 2-2.5 units pH between the intracellular compartment (approx. pH 7.2) and urine limiting removal of protons. The excretion of protons in unbuffered urine would thus require approx 200 liters of urine. Titratable acids (mainly phosphate) buffer protons and urine. A major fraction of protons, however, is excreted bound by ammonia after parallel secretion into urine (approx. 2/3 of the daily acid load). Ammonia secretion occurs along the entire length of the collecting duct system [3].

Ammonia is generated from metabolism of glutamine in the proximal tubule regenerating bicarbonate lost during metabolism [4-5]. Ammonium is secreted into urine, reabsorbed in the thick ascending limb, and accumulated in the interstitium [3]. The high interstitial concentration and the tissue-urine pH gradient drive ammonia excretion by intercalated cells. Intercalated and principal cells both contribute to ammonia secretion into urine [1].

According to Overton's rule [6], gases as CO_2 or NH_3 are thought to move easily across biological membranes via diffusion. In 1945, the great renal physiologist Robert Pitts had described the role of ammonium in renal acid secretion and postulated that ammonium secretion is a passive process driven solely by the ammonia concentration gradient, via non-ionic diffusion of ammonia across the luminal membrane, and the following trapping of ammonium in urine after protonation [7]. This hypothesis had remained textbook knowledge until recently.

Gas transport by Rhesus proteins

Ammonia and ammonium are transported by a family of proteins in plants, bacteria, and yeast. In 2000 it was noticed that the mammalian homologues were also able to mediate transport and that these proteins belonged to the family of Rhesus proteins [8]. The mammalian Rhesus proteins RhAG, RhBG and RhCG induce NH_3 or NH_4^+ transport in various heterologous cell models even though the exact transport mode and substrate (i.e. NH_3 or NH_4^+) as well as the coupling to other ions (i.e. counter- or cotransport of protons) and stoichiometry have remained controversial [3]. The algae Rh1 protein might even be involved in CO_2 permeability. In mammals, RhAG is detected in erythrocytes, RhBG in liver, kidney, and ovary, and RhCG in kidney, liver, brain, skeletal muscle, prostate, and pancreas [3].

In kidney, RhBG and RhCG have been found exclusively in the distal tubule, connecting tubule, and cortical and medullary collecting duct [3]. RhBG was detected in rodent kidney on the basolateral side of intercalated and principal cells. It has not been detected in human kidney to date. In contrast, RhCG is localized to the apical and basolateral membrane of intercalated and principal cells [3, 9]. During acidosis

the cortical collecting duct becomes a major site of ammonia secretion [10] paralleled by the strongest staining for RhCG.

RhCG is a novel NH_3 gas channel in kidney

Different mouse models have been generated to examine the role of Rhesus proteins in vivo and to clarify a role in ammonia or ammonium transport. RhAG KO show greatly diminished ammonia fluxes in erythrocytes resembling patients with inherited disorders of the red blood cells rhesus complex. RhBG deficient mice had normal urinary ammonium excretion and unaltered basolateral NH_3/NH_4^+ permeabilities as well as transepithelial ammonia fluxes in the collecting duct [11]. In contrast, genetic deletion of RhCG in at least three different mouse models demonstrates a critical role for this protein in urinary ammonium excretion [12-13]. RhCG KO show only mildly reduced ammonium excretion under basal conditions and normal blood acid-base parameters. However, acid-loading mice with HCl or NH_4Cl induces more severe metabolic acidosis and KO mice have a strongly reduced maximal capacity to increase urinary ammonium excretion. After prolonged acid-loading (7 days) also heterozygous RhCG mice develop a more pronounced acidosis with lower urinary ammonium excretion suggesting haploinsufficiency. Ammoniagenesis in the proximal tubule is preserved in RhCG KO mice. We proceeded to the cellular level and performed microperfusion experiments in the cortical and outer medullary collecting ducts from acid-loaded mice. Ammonia but not ammonium permeability of the apical membrane is reduced by about 60 - 80 %. Similarly, when we measured total transepithelial NH_3 permeability, we found an 80 % reduction [12]. Since RhCG is expressed on the baoslateral membrane, we also tested the possibility that RhCG may play a role in the uptake of ammonium from the interstitium and found also there a reduction of NH_3 permeability by about 30-50 %. In heterozygous mice an intermediate phenotype was found. Thus, RhCG is critical for urinary ammonium excretion and is required for collecting duct NH_3 secretion.

Functional and structural data suggest that RhCG and related Rhesus proteins function as gas channels. Heterologously expressed RhCG interacts with both NH_3

and NH_4^+. Our in vitro microperfusion experiments are consistent with a role in NH_3 fluxes but cannot rule out other transport modes. RhAG in human and mouse red blood cells mediates NH_3 fluxes . Reconstituted human RhCG in liposomes induces NH_3 but not NH_4^+ fluxes [14-15]. Finally, crystal structures from the *E. coli* homologue AmtB as well as from human RhCG show a vestibule gated by phenylalanines and a pore region lined by histidine residues which would exclude charged molecules such as NH_4^+ and allow only the passage of a neutral NH_3 [14]. Collectively these data demonstrate that RhCG and other family members are not only subunits of the permeation pathway but form the pore of the transporter/channel and mediate the passage of the gas NH_3.

In summary, RhCG forms a gas channel required for normal ammonium excretion by mediating NH_3 fluxes. Non-ionic diffusion, as postulated by Pitts, does not account for the majority of NH_3 excretion. Thus, rhesus proteins like RhCG represent an novel class of membrane proteins allowing the passage of a gas contradicting the rule of Overton.

Proton-activated receptors

Coordinated adaptation of ventilation during metabolic acidosis is a major compensatory mechanism to regulate systemic acid-base homeostasis. The initial trigger for this adaptive increase in ventilation is a rise in arterial CO_2 partial pressure (pCO_2) and/or a fall in arterial blood pH. These changes are sensed at two distinct sites, the carotid body and different regions of the ventral medulla oblongata. Upon stimulation of chemosensitive cells in these regions, ventilation (primarily tidal volume and secondary also respiratory frequency) are increased [16-18]. Accumulating evidence suggests that not changes in pCO_2 but in local pH are sensed but it remains controversially discussed if changes in intra- or extracellular pH are primarily sensed. The molecules mediating the sensing of pCO_2 or pH, however, have not been identified to date. The candidate proteins include several ion channels (TASK, ASIC), the sodium/proton exchanger NHE3, and intracellular signaling molecules that are sensitive to changes in pH. However, studies in gene ablated mice or in pharmacologically treated animal models did not yield convincing evidence for the role of any of these candidates to date.

G protein-coupled receptors (GPCR) recognize a variety of ligands including hormones, biogenic amines, amino acids, lipids, metabolites, and also small molecules such as calcium. Evidence also suggests that mechanisms exist by which cells can sense changes in extracellular pH but it has remained controversial if receptors or other types of membrane proteins such as pH-sensitive ion channels act as primary sensors.

The GPCRs OGR1 (Ovarian Cancer G protein-coupled receptor 1), GPR4, and TDAG8 (T-cell death associated gene 8) were initially described as receptors for lipids [19]. Subsequently, Ludwig et al found that not lipids were acting on these receptors but that small changes in extracellular pH were eliciting the production of cAMP in the case of GPR4 and TDAG8 and caused a rise in intracellular calcium and IP_3 concentrations in OGR1 expressing cells [19-20]. Activation of all three receptors occurs in the physiologic pH range with almost no activity around pH 7.8 and full activation around pH 6.8 with half-maximal activation between pH 7.5 and 7.3. Expression of OGR1 and GPR4 is found in most organs, whereas TDAG8 appears to be more restricted to immune cells. More recently, these receptors have been implicated in a variety of functions such as osteoclast activation during acidosis, modulation of immune cells during inflammation, regulation of endothelial cell function, tumor growth, and pain perception or transduction. Since all these functions were mostly deduced from in vitro experiments, it remains to be established in *in vivo* or *ex vivo* experiments if these receptors indeed contribute to these processes.

GPR4 is critical for CO_2 and acidosis stimulated ventilation

We used mice lacking GPR4 and examined their systemic acid-base parameters under basal conditions. Mice had lower blood pH and mild acidosis with slightly reduced urinary ammonium excretion. However, the most striking finding was that arterial partial pressure of CO_2 was elevated suggesting that at least part of the acidosis was due to impaired ventilation. Since lung volume and histology was unremarkable, awake mice were examined for their ability to adapt breathing to two types of challenges, changes in ambient CO_2 (acute hypercapnia) and metabolic acidosis (induced by 24 hrs of NH_4Cl loading). Basal tidal volume was lower in GPR4 KO mice. Acute exposure to CO_2 increased tidal volume and respiratory frequency in wildtype mice whereas GPR4 increased also both parameters but tidal volume did

not reach the same level as in wildtype mice. In contrast, adaptation to altered ambient oxygen is fully preserved in GPR4 KO mice.

We tried to dissect where GPR4 acts on the control of ventilation by denervating the carotid body in mice. In denervated wildtype mice, acute hypercapnia increased tidal volume and respiratory frequency whereas in denervated GPR4 KO mice no increase in tidal volume could observed. Taken together, it appears that GPR4 contributes partially to ventilatory control in carotid bodies but is critical for normal adaptation of ventilation in the medulla oblongata. The exact role of GPR4, its regulation and down-stream targets, however, remain to be identified.

Summary

Systemic acid-base homeostasis is influenced by many variables and controlled by different organs. The molecular control mechanisms, however, have only begun to emerge recently. Here, we described two membrane proteins with unusual properties. RhCG is a member of the first mammalian gas channel family mediating the transfer of NH_3. GPR4 together with two other G protein-coupled receptors forms a small subfamily of receptors that appear to be involved in sensing extracellular proton concentrations and regulating systemic (and local) pH homeostasis.

Acknowledgements

This work would not have been possible without the contribution of many co-workers and collaborators. I would like to thank particularly, Soline Bourgeois (Zurich), Pascal Houillier (Paris), Erik Christensen (Aarhus), Olivier Devuyst (Brussels), Ana Velic (Zurich, now Tubingen), Klaus Seuwen (Basel, Novartis), and Jorge Soliz (Zurich). The generous support from the 6th and 7th EU Frame work projects EuReGene and EUNEFRON as well as by the Swiss National Science Foundation is gratefully acknowledged.

Questions posées lors de la séance du samedi 19 juin 2010 à M. le Prof. C. WAGNER.

M. O. Devuyst. – The results of the denervation experiments are particularly interesting, as they suggest that there are compensatory mechanisms operating in the knock-out mice. Do you have an idea about these mechanisms? Thank you very much for your beautiful lecture.

M. C. Wagner. – This is an important question. Indeed, several candidate proteins have been suggested that could mediate chemosensing in the carotid body and brain stem, including several potassium channels from the TASK family, the proton-activated sodium channels from the ASIC family, or the sodium/proton exchanger NHE3. However, evidence for an important role of any of these proteins in pH-sensing is missing to date. Our results with the denervated animals suggest that GPR4 plays a critical role in pH-sensing in the brain stem, whereas in the carotid body other mechanisms may be operating. In patients with rare bilateral tumors of the carotid body, paragangliomas, removal of both carotid bodies leads to a loss of a rapid component of adaptation to hypercapnia whereas a slower component is fully preserved. Again, this indicates that possibly different mechanisms underlie peripheral and central chemosensitivity also in humans. At present it is very difficult to dissect these mechanisms on a cellular and molecular level since we do not know which cells exactly mediate chemosensitivity and how the signal(s) is perceived and transduced.

M. J.E. Dumont. – The GPR4 regulation puzzles me. The receptors we work with are on/off receptors on when the ligand is bound.

Where you have an optimum – how do you visualize this structure (s) ?

M. C. Wagner. – This is very open and interesting question. The structure of the receptor has been modeled based on the known structure of rhodopsin [19-20]. This model structure predicts the presence of a cluster of histidine residues located where normally the “binding pocket” of the ligand would be. These histidines are most likely protonated and lead to the activation of the receptor. Mutations of these shift the activation of the receptor to higher proton concentrations. As for the regulation of these receptors: many classic G protein coupled receptors undergo some kind of desensitivation upon binding of their ligand. The classic example is the adrenergic receptor that is internalized after binding of adrenaline or similar agonists in a β-arrestin dependent manner and recycles back to the membrane to some extent. There are other relevant examples that lack this internalization upon activation by agonists. The calcium-sensing receptor of the parathyroid gland does not undergo

such an internalization. One might speculate that this makes sense teleologically, since calcium is not an on/off signal but is always present in the extracellular fluids – like protons. Indeed some preliminary evidence suggests that OGR1 or GPR4 do not desensitize and internalize upon activation, but this field requires certainly more work and hard data.

References

1. WAGNER C. A., O. DEVUYST, S. BOURGEOIS, and N. MOHEBBI, *Regulated acid-base transport in the collecting duct.* Pflugers Arch, 458, 137-56 (2009)
2. WAGNER C. A., FINBERG, K E, BRETON, S, MARSHANSKY, V, BROWN, D, GEIBEL, J P, *Renal vacuolar H^+-ATPase.* Physiol Rev, 84, 1263-314 (2004)
3. WEINER I. D. and L. L. HAMM, *Molecular mechanisms of renal ammonia transport.* Annu Rev Physiol, 69, 317-40 (2007)
4. CURTHOYS N. P., *Renal ammonium ion production and excretion*, in *Seldin and Giebisch's The Kidney. Physiology and Pathophysiology.*, R.J. Alpern and S.C. Hebert, Editors. 2008, Elsevier. 1601-1619.
5. BUSQUE S. M. and C. A. WAGNER, *Potassium restriction, high protein intake, and metabolic acidosis increase expression of the glutamine transporter SNAT3 (Slc38a3) in mouse kidney.* Am J Physiol Renal Physiol, 297, F440-50 (2009)
6. MISSNER A. and P. POHL, *110 years of the Meyer-Overton rule: predicting membrane permeability of gases and other small compounds.* Chemphyschem, 10, 1405-14 (2009)
7. PITTS R. F., *The Renal Regulation of Acid Base Balance with Special Reference to the Mechanism for Acidifying the Urine. Ii.* Science, 102, 81-85 (1945)
8. MARINI A. M., MATASSI, G, RAYNAL, V, ANDRE, B, CARTRON, J P, CHERIF-ZAHAR, B, *The human Rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast.* Nat Genet, 26, 341-4 (2000)
9. BROWN A. C., D. HALLOUANE, W. J. MAWBY, F. E. KARET, M. A. SALEEM, A. J. HOWIE, et al., *RhCG is the major putative ammonia transporter expressed in the human kidney, and RhBG is not expressed at detectable levels.* Am J Physiol Renal Physiol, 296, F1279-90 (2009)
10. SAJO I. M., M. B. GOLDSTEIN, H. SONNENBERG, B. J. STINEBAUGH, D. R. WILSON, and M. L. HALPERIN, *Sites of ammonia addition to tubular fluid in rats with chronic metabolic acidosis.* Kidney Int, 20, 353-8 (1981)
11. CHAMBREY R., D. GOOSSENS, S. BOURGEOIS, N. PICARD, M. BLOCH-FAURE, F. LEVIEL, et al., *Genetic ablation of Rhbg in the mouse does not impair renal ammonium excretion.* Am J Physiol Renal Physiol, 289, F1281-90 (2005)
12. BIVER S., H. BELGE, S. BOURGEOIS, P. VAN VOOREN, M. NOWIK, S. SCOHY, et al., *A role for Rhesus factor Rhcg in renal ammonium excretion and male fertility.* Nature, 456, 339-43 (2008)
13. LEE H. W., J. W. VERLANDER, J. M. BISHOP, P. IGARASHI, M. E. HANDLOGTEN, and I. D. WEINER, *Collecting duct-specific Rh C glycoprotein deletion alters basal and acidosis-stimulated renal ammonia excretion.* Am J Physiol Renal Physiol, 296, F1364-75 (2009)
14. GRUSWITZ F., S. CHAUDHARY, J. D. HO, A. SCHLESSINGER, B. PEZESHKI, C. M. HO, et al., *Function of human Rh based on structure of RhCG at 2.1 A.* Proc Natl Acad Sci U S A, 107, 9638-4 (2010)
15. MOURO-CHANTELOUP I., S. COCHET, M. CHAMI, S. GENETET, N. ZIDI-YAHIAOUI, A. ENGEL, et al., *Functional reconstitution into liposomes of purified human RhCG ammonia channel.* PLoS One, 5, e8921 (2010).
16. MULKEY D. K., R. L. STORNETTA, M. C. WESTON, J. R. SIMMONS, A. PARKER, D. A. BAYLISS, et al., *Respiratory control by ventral surface chemoreceptor neurons in rats.* Nat Neurosci, 7, 1360-9 (2004)
17. LAHIRI S., FORSTER, R E 2ND, *CO_2/H^+ sensing: peripheral and central chemoreception.* Int J Biochem Cell Biol, 35, 1413-35 (2003)
18. FELDMAN J. L., G. S. MITCHELL, and E. E. NATTIE, *Breathing: rhythmicity, plasticity, chemosensitivity.* Annu Rev Neurosci, 26, 239-66 (2003)

19. SEUWEN K., M. G. LUDWIG, and R. M. WOLF, *Receptors for protons or lipid messengers or both?* J Recept Signal Transduct Res, 26, 599-610 (2006)
20. LUDWIG M. G., VANEK, M, GUERINI, D, GASSER, J A, JONES, C E, JUNKER, U, HOFSTETTER, H, WOLF, R M, SEUWEN, K, *Proton-sensing G-protein-coupled receptors.* Nature, 425, 93-8 (2003)